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Polyphenolic Compounds and Antimicrobial Activity of *Quercus aucheri* Leaves

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Chromatographic studies (CC, VLC, MPLC, and PTLC) on ethyl acetate extract from the leaves of *Quercus aucheri* yielded 2 flavonoids (quercetin 3-*O*-α-L-arabinopyranoside (**1**), quercetin 3-*O*-β-D-galactopyranoside (**2**)) and 2 tannin precursors and a procyanidin [(isolated as peracetates of (+)-catechin (**3a**), (+)-gallocatechin (**4a**) and epicatechin-(4β →8)-catechin (**5a**))]. The structures of the compounds were elucidated by UV, 1D-NMR (^{1}H, ^{13}C, TOCSY) and 2D-NMR (COSY, HSQC, HMBC) techniques. Different extracts (80% MeOH, EtOAc,*n*-BuOH and H_2O) from the leaves of *Q. aucheri* were investigated for their antimicrobial activity against 2 Gram-positive and 2 Gram-negative bacteria and 3 yeast-like fungi by a broth microdilution method. EtOAc extract, which showed the highest antimicrobial activity, was further used for isolation.

Key Words: Quercus aucheri, flavonol glycosides, (+)-catechin, (+)-gallocatechin, epicatechin-(4β →8)-catechin, antimicrobial activity.

Introduction

Quercus aucheri Jaub. & Spach (Fagaceae) is one of the endemic plants of the 18 *Quercus* species that grow in Turkey[1]. *Quercus* (oak) bark and galls are used as an astringent, antiseptic and hemostatic. A decoction of *Quercus* is also used to treat acute diarrhea and inflammation. Moreover, the decoction of these plants could be used for burns and cuts[2−4]. The present study was undertaken to evaulate the antimicrobial activity of different extracts and to chemically investigate the most active part of the extracts from *Q. aucheri* leaves.

Experimental

General Experimental Procedures: The UV spectra were recorded on a Hitachi HP 8452 A spectrophotometer. Optic rotations were recorded on a Rudolph Autopol IV polarimeter. NMR measurements at room

temparature were measured using Bruker AMX 300 and Bruker AMX 600 spectrometers (^{1}H: 300.13 and 600 MHz; ^{13}C: 150 MHz). Negative-mode ESIMS were recorded on a Finnigan TSQ 7000 instrument. Sephadex LH 20 (Pharmacia) columns (3.5 x 40 cm and 2.5 x 44 cm) were used for open column chromatographic separations. MPLC was performed on a Büchi (2.5 x 45 cm) glass column packed with Europrep RP-18 (20-45 μ), using a Büchi B-684 pump. VLC separation was realized on a glass column (2.5 x 15 cm) packed with Europrep RP-18 (20-45 μ). TLC analyses were carried out on silica gel 60 F_{254} precoated plates (Merck, Darmstadt, Germany), and detection was performed with 1% vanilin/H_2SO_4.

Plant material: *Q. aucheri* leaves were collected in August 2002 from Gözne, Mersin province, Turkey. A voucher specimen (HUEF 02019) was deposited at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and isolation: Dried and powdered *Q. aucheri* leaves (800 g) were extracted with a MeOH:H_2O (8:2) mixture (4 x 3500 mL). The extract was suspended in water and partitioned with petroleum ether (40-60°) (7 x 300 mL), ethyl acetate (5 x 400 mL) and *n*-butanol (4 x 150 mL), in that order. This procedure yielded 209.27 g of MeOH extract, 0.98 g of petroleum extract, 34.66 g of EtOAc extract, 3.63 g of *n*-BuOH extract, and 170 g of H_2O extract.

A portion of the EtOAc extract (15.9 g) was subjected to a Sephadex LH-20 column using 96% ethanol to give 6 main fractions, A_1-A_6. Fr. A_3, which contains compounds **1**, **2** and **3**, was reapplied to the Sephadex LH-20 column utilizing the same solvent system to yield 5 subfractions, $A_3$1-5. Fr. $A_3$3 (558 mg) was rechromatographed over RP-18 MPLC to give **1** (15 mg, eluted with 45% MeOH), **2** (13 mg, eluted with 45% MeOH), and **3** (68 mg, eluted with 20% MeOH). Then 50 mg of **3** was acetylated with Ac_2O, yielding 102 mg of **3** peracetate derivative (**3a**). Purification of fr. A_4 by Sephadex LH-20 CC using 96% ethanol yielded 7 subfractions, $A_4$1-7. Fr. $A_4$3 was similarly applied to RP-18 MPLC using 20% MeOH to obtain impure **4**. Compound **4** was acetylated with Ac_2O and purified by preparative TLC (silica gel 60 F_{254}) (benzene:acetone 8:2) to give 6 mg of pure **4** acetate (**4a**). Fr. A_6 (172 mg) was also chromatographed on the Sephadex LH-20 column, eluting with 96% ethanol, to give frs. $A_6$1-4. Fr. $A_6$3 (21 mg) was rechromatographed on RP-18 VLC, eluting with 17.5% MeOH, to yield compound **5**. This fraction was acetylated with Ac_2O to give the peracetate derivative of compound **5** (**5a**) (7 mg).

Antimicrobial activity method: The broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was used to determine the antimicrobial activity[5−6]. The test was performed in Mueller-Hinton broth (MHB, Difco Laboratories, Detroit, MI, USA) for antibacterial studies. For yeast-like fungi, RPMI-1640 medium with L-Glutamine buffered with MOPS buffer (ICN-Flow, Aurora, OH, USA) was used. The inoculum densities were approximately 5 x 10^5 and 0.5-2.5 x 10^5 cfu/mL for bacteria and fungi, respectively. Ampicillin and fluconazole were used as reference antibiotic powder against bacteria and fungi, respectively.

Quercetin 3-*O*-α-L-arabinopyranoside (**1**): $[\alpha]_D^{20}$ -53.96^o (MeOH; c 1). UV $\lambda_{max.}$nm (MeOH): 256.0, 269.0 sh, 305.0 sh, 359.0; (NaOMe): 272.5, 281.0 sh, 330.5, 411.5; (NaOAc): 267.0, 297.0 sh, 374.0; (NaOAc+H_3BO_3): 262.0, 303.0 sh, 375.5; ($AlCl_3$): 272.0, 303.0 sh, 433.5; ($AlCl_3$+HCl): 268.5, 304.0 sh, 347.5, 410.0. ^{1}H-NMR (600 MHz, CD_3OD): δ 7.77 (1H, *d*, *J*1.5 Hz, H-2′), 7.61 (1H, *dd*, *J* 1.5/8.0 Hz, H-6′), 6.90 (1H, *d*, *J* 8.0 Hz, H-5′), 6.42 (1H, *d*, *J*1.5 Hz, H-8), 6.23 (1H, *d, J 1.5* Hz H-6), 5.18 (1H, *d*, *J* 6.6 Hz, H-1′), 3.92 (1H, *dd*, *J* 7.6.6/9.0 Hz H-2′), 3.85 (1H, *d*, *J* 11.7/3.0 Hz, H-5″a), 3.84 (1H, *m*, H-4′), 3.67 (1H,

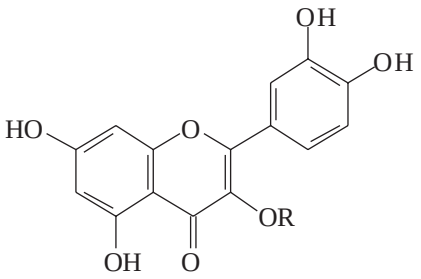
dd, *J* 3.0/9.0 Hz, H-3′), 3.47 (1H, *d*, *J* 11.7/1.8 Hz, H-5″b). Negative-ion ESIMS: m/z 433 $[M\text{-}H]^-$

Quercetin 3-*O*-β-D-galactopyranoside (**2**): $[\alpha]_D^{20}$-14.99^o (MeOH; c 1). UV $\lambda_{max.}$ nm (MeOH): 255.0, 270.0 sh, 304.0 sh, 362.5; (NaOMe): 270.0, 328.5, 409.5; (NaOAc): 267.0, 367.5; (NaOAc+H_3BO_3): 261.0, 304.0 sh, 379.0; ($AlCl_3$): 269.0, 305.0 sh, 428.5; ($AlCl_3$+HCl): 266.5, 300.0 sh, 362.5 sh, 402.5. ^{1}H-NMR (600 MHz, CD_3OD): δ 7.87 (1H, *d*, *J* 1.5 Hz, H-2′), 7.61 (1H, *dd*, *J* 1.5/8.0 Hz, H-6′), 6.89 (1H, *d*, *J* 8.0 Hz, H-5′), 6.42 (1H, *d*, *J*1.5 Hz, H-8), 6.23 (1H, *d*, *J*1.5 Hz, H-6), 5.20 (1H, *d*, *J* 6.6 Hz, H-1′), 3.88 (1H, *brs*, H-4″), 3.85 (1H, *t*, *J* 8.7 Hz, H-2″), 3.67 (1H, *dd*, *J* 10.8/5.4 Hz, H-6′a), 3.59 (2H, *m*, H-3″/6″b), 3.51 (1H, *d*, *J* 5.4 Hz, 5″), Negative-ion ESIMS: m/z 463 $[M\text{-}H]^-$

(+)-Catechin penta-acetate (**3a**): $[\alpha]_D^{20}$ +33.98° (acetone, c 1). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 2.00 (3H, *s*, OAc at C-3), 2.27-2.28 (12H, *brs*, 4 OAc), 2.66 (1H, *dd*, *J* 6.0/16.8 Hz, Hc′), 2.87 (1H, *dd*, *J* 4.8/16.8 Hz, Hc), 5.14 (1H, *d*, *J* 6 Hz, Ha), 5.25 (1H, *m*, Hb), 6.60 (1H, *d*, *J* 2.5 Hz, H-6), 6.66 (1H, *d*, *J*2.5 Hz, H-8), 7.16-7.25 (3H, *m*, H-2′/5′/6′). ^{13}C-NMR: (150 MHz, $CDCl_3$): δ 20.7-21.2 (-OCO<u>C</u>H$_3$), 23.9 (C-4),68.3 (C-3), 77.7 (C-2), 106.4 (C-8), 107.7 (C-6), 110.2 (C-10), 121.8 (C-2′),123.7 (C-5′), 124.4 (C-6′), 136.2 (C-1′),142.1 (C-3′/4′), 149.4 (C-5), 149.9 (C-7), 154.4 (C-9), 168.1-170.1 (-O<u>C</u>OCH$_3$).

(+)-Gallocatechin hexa-acetate (**4a**): $[\alpha]_D^{20}$ +5.0° (acetone, c 1). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.02 (3H, *s*, OAc at C-3), 2.27-2.29 (15 H, *brs*, 5 OAc), 2.67 (1H, *dd*, *J* 6.9/16.7 Hz, Hc′), 2.91 (1H, *dd*, *J* 5.4/16.7 Hz, Hc), 5.12 (1H, *d*, *J* 6.6 Hz, Ha), 5.21 (1H, *m*, Hb), 6.61 (1H, *d*, *J* 2.1 Hz, H-6), 6.66 (1H, *d*, *J* 2.1 Hz, H-8), 7.12 (2H, *s*, H-2′/6′).

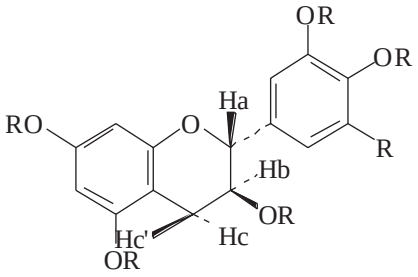
Epicatechin-(4β→8)-catechin deca-acetate (**5a**): $[\alpha]_D^{20}$ -24.98° (acetone, c 1). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.87-2.37 (30H, *m*, 10 OAc), 2.57 (1H, *dd*, *J* 9.1/16.7, Hf′), 3.22 (1H, *dd*, *J* 6.7/16.7, Hf), 4.36 (1H, *d*, *J* 10.7 Hz, Hd), 4.45 (1H, *d*, *J* 2.0 Hz, Hc), 5.08 (1H, *ddd*, He), 5.16 (1H, *brs*, Hb), 5.48 (1H, *brs*, Ha), 6.03 (1H, *d*, *J* 2.1 Hz, H-6), 6.33 (1H, *d*, *J* 2.1 Hz, H-8), 6.71 (1H, *s*, H-6′), 6.92-7.30 (6H, *m*, H-2′,5′,6′/H-2‴,5‴,6‴).



1 R= α-L-arabinopyranose

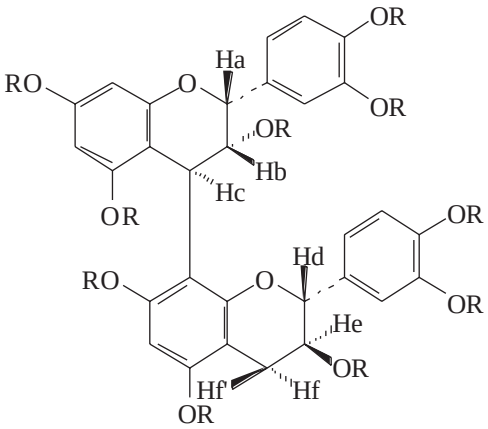
2 R= β-D-galactopyranose

Figure 1



3 R= H **3a** R=Ac
4 R= OH **4a** R=Ac

Figure 2



5 R=H **5a** R=Ac

Figure 3

Results and Discussion

From the EtOAc extract, 2 flavonol glycosides, quercetin 3-*O*-α-L-arabinopyranoside (**1**) and quercetin 3-*O*-β-D-galactopyranoside (**2**), and tannin precursors (+)-catechin (**3**), gallocatechin (**4**) and a procyanidin epicatechin-(4β $\rightarrow$8)-catechin (**5**) as peracetates (**3a**, **4a**, **5a**) were isolated. The structures of these compounds were identified using spectroscopic [UV, 1D (^{1}H, ^{13}C, TOCSY), 2D-NMR (COSY, HSQC, HMBC) and polarimeter] and chemical (acetylation) evidence as well as, comparison with the published data[7−11].

The EtOAc extract from the leaves of *Q. aucheri* was the most active extract against all the tested microorganisms (see Table). This extract showed high activity against all tested fungi (MIC = 1.2 μg/mL for *C. parapsilosis,* and 2.4 μg/mL for *C. albicans*) and a Gram-positive bacterium *S. aureus* with a MIC value of 2.4 μg/mL. Therefore, the compounds in EtOAc extract have to be studied for their characterization.

Table. Antimicrobial activity of *Q. aucheri* leaf extracts.

Test Materials	MIC (μg/mL)						
	Bacteria				Fungi		
	S. aureus ATCC 29213	E. faecalis ATCC 29212	E. coli ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019
MeOH extract	156.2	625.0	156.2	39.0	4.9	9.7	2.4
EtOAC extract	2.4	19.5	156.2	39.0	2.4	4.9	1.2
n-BuOH extract	312.5	312.5	312.5	39.0	4.9	4.9	1.2
H_2O extract	39.0	>1250	312.5	312.5	1250	156.2	1250
Ampicillin	0.2	0.5	4	-			
Fluconazole					1	32	4

(-): No inhibition

Acknowledgments

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